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MULTIPLE SCREENING OF CORN INTRODUCTIONS

for

Resistance to Diseases and Insects

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MULTIPLE SCREENING OF CORN INTRODUCTIONS FOR RESISTANCE TO DISEASES AND INSECTS

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SUMMARY

A method of multiple inoculation of a large number of corn accessions with several pathogens and an insect pest in a successive sequence is described. Genotypic sources of corn resistant to the following diseases and insect can be detected in the same set of tests: Smut (Ustilago maydis (DC.) Cda.), several races of leaf rust (Puccinia sorghi Schw.), leaf blight (Helminthosporium turcicum Pass.), Diplodia and Fusarium stalk rots (Diplodia zeae (Schw.) Lév. and Fusarium spp.), and the European corn borer (Ostrinia nubilalis (Hübner)). Plants with genes having multiple resistance are also detectable.

INTRODUCTION

To locate genes for resistance, many screening projects are underway. However, collections of early and even recent introductions remain to be systematically screened. Of approximately 10,000 corn accessions introduced in the past 60 years, there are viable seeds of about 5,000. The screening of such a large number for several important corn diseases and insects, when done by conventional methods, is cumbersome. Even over a long period, the appearance of new pathogens, new races of pathogens, or new insects can necessitate frequent rescreening of all material. New and more rapid techniques are required. Furthermore, in searching for sources of multiple resistance, it is necessary to screen the same species for resistance to several pathogens and insects simultaneously.

In 1958-64, at the North Central Regional Plant Introduction Station, Ames, Iowa, a combined method was developed involving the following diseases and insect pest: Smut (Ustilago maydis (DC.) Cda.), leaf rust (Puccinia sorghi Schw.), leaf blight (Helminthosporium turcicum Pass.), stalk rots (Diplodia zeae (Schw.) Lév. and Fusarium spp.), and the European corn borer (Ostrinia nubilalis (Hübner)).

Notes were taken on other diseases that developed from natural infections. Also some nonpathogenic inherited factors that cause viruslike symptoms on leaves were classified and rated according to disease reactions. One such genetic malady, tentatively called yellow mottle of corn, has been previously reported (Leppik, 1962)[2] and is illustrated in figure 1.

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[1] This method was elaborated for the screening of corn introductions during the author's assignment as research plant pathologist, North Central Regional Plant Introduction Station, and professor, Iowa State University, Ames. The manuscript was prepared subsequently in the series of Plant Introduction Investigation Papers as Report No. 6 in Beltsville, Md. H. L. Hyland, A. J. Oakes, A. L. Robert, W. Q. Loegering, and W. H. Skrdla made several suggestions.

[2] The year after the author's name is the key to the reference in Literature Cited at the end of this report.

Figure 1.--Heritable yellow mottle on leaf in translucent (left) and reflected light (right).

DESCRIPTION OF METHOD

Field Inoculations

For multiple inoculations, an irrigation system was provided for sprinkling the plants before inoculation. Each corn accession was planted in a single row of 100 plants (fig. 2). Each row was divided at midpoint by a furrow, on each side of which were 50 plants. One part of each row (left or right) was inoculated in sequence with smut, leaf rust, leaf blight, Diplodia stalk rot, Fusarium stalk rot, and the corn borer. The remaining part of each row was used as a check.

About 200 to 300 accessions may be handled easily by a team of five or six field workers. To make inoculations successively with the five pathogens and the insect, from 6 to 8 hours of teamwork are needed weekly throughout the growing season. The inoculation schedule must conform with the natural appearance of pathogens and the insect in the field.

Figure 2.--Arrangement of inoculation lots in field. Numbered accessions (P.I.), 100 plants each, are divided by a furrow into two equal parts, one for inoculation (I) and another for check (C) in the following sequence: Left, smut (I), leaf rust (C), leaf blight (I), Diplodia stalk rot (C), Fusarium stalk rot (I), corn borer (C); right, smut (C), leaf rust (I), leaf blight (C), Diplodia stalk rot (I), Fusarium stalk rot (C), corn borer (I).

Greenhouse Tests

For detailed study of resistance, all promising and resistant accessions detected during the multiple field screening must be retested in the greenhouse. There the reactions of plants to specific pathogens, pathogenic races, and the insect pest are tested individually in isolated compartments.

Evaluations

Evaluations should be performed by an experienced pathologist or well-trained technician. They may be expressed as numerical ratings (0 = none, 5 = maximum infection, if the scale 0-10 is not preferred.) Ratings made on a scale 0-9 can be used on an electronic computing machine card. The number or area of lesions on a leaf and the proportion of infected leaves on a plant can be used as a basis for blight ratings. Stalk rots are rated according to the relative areas of decay in stalks (Hooker and Russell, 1962).

Smut

For field inoculations with smut, aqueous suspensions of about 1 million viable spores per milliliter are commonly used, with 0.1 percent Tween 20[3] added. Plants are sprayed with this suspension to the point of runoff. One or two sprayings are necessary before tasseling.

However, soil inoculation appears to be the most practical method of smut inoculation. At Ames, smut galls were collected in the field, kept overwinter in an unheated room, and used as inoculum the next spring. One evaluation was made before tasseling and one afterward. In fields planted to corn for several years in succession, the soil became heavily infested with spores, and the tests became relatively dependable.

Leaf Rust

Leaf rust inoculations in the field are highly dependent on the weather. Since water droplets on leaves are necessary for successful germination of spores, field inoculations should be made in the late afternoon or immediately after rain or sprinkling. Rain immediately after inoculation may wash off most of the spores before they can germinate.

Sprinkling appeared to be necessary in all leaf rust inoculations at Ames, both in the field and the greenhouse. Field inoculations made without prior sprinkling resulted in few rust pustules, which were difficult to rate, and many pustules formed when inoculations were made after sprinkling (fig. 3).

Inoculum was prepared immediately before each inoculation. Rust spores (urediospores) were collected by vacuum pump from rust-infected greenhouse plants during the winter and stored at 34° F. until needed. Spore suspensions of all endemic rust races were prepared in distilled water and thoroughly mixed in an electric blender. Races R_1 through R_6 were used for greenhouse testings. The germinability of spores was determined in a droplet of water by microscopic examination. Since most spores germinate readily, the spore suspension should be used for inoculation as soon as it is prepared.

[3] Trade names are used in this publication solely to provide specific information. Mention of a trade name does not constitute a guarantee or warranty by the U.S. Department of Agriculture and does not signify that the product is approved to the exclusion of other comparable products.

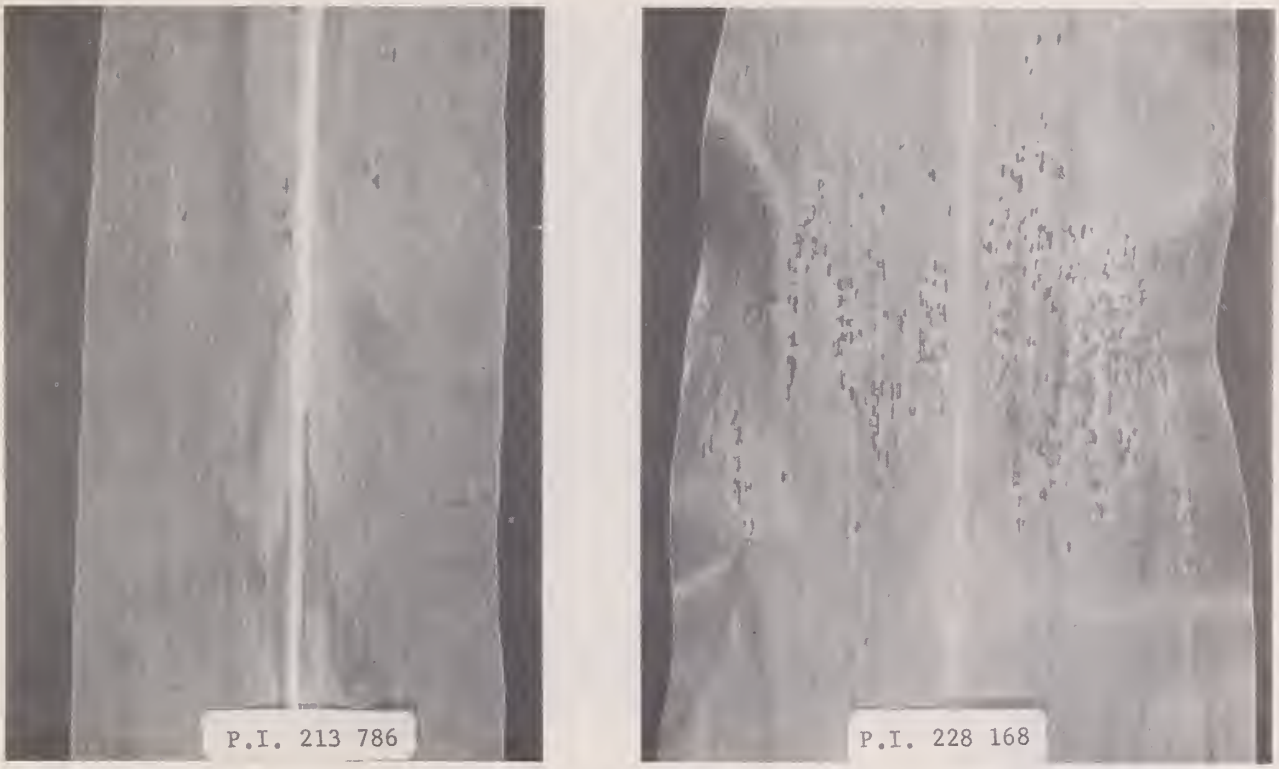

Figure 3.--Field inoculation made without sprinkling when only a few rust pustules, difficult to rate, formed (left) and after sprinkling when heavy infection with numerous rust pustules resulted (right).

Field Inoculation

The plants were first inoculated at the three-leaf stage. The first three or four plants in each row were sprayed with the inoculum, using high-pressure hand sprayers (fig. 4). To obtain maximum infection, successive inoculations of three or four additional plants per row were made each week until all plants in one-half of every row had been sprayed once or twice.

Leaf rust resistance was evaluated several times, both before and after tasseling. Relative earliness of maturity helps to determine the time of inoculation. The inoculated half of each accession was compared with the uninoculated half; the latter showed an average grade of natural rust infection. No differences were exhibited by susceptible varieties during treatment, except in the years when rust development was low and when uninoculated plants remained rust-free. Rust-free plants were retested in the greenhouse during the winter and again in the field the following year.

Since the degree of resistance of seedlings sometimes differs from the resistance of old plants, seedlings of promising accessions must be tested in the greenhouse. However, multiple screening in the field helps to reduce the greenhouse work to those promising accessions that merit further study.

Greenhouse Inoculation

Usually only seedlings are inoculated in the greenhouse. Therefore instead of spraying, as in the field, a medical syringe was used to inject a spore suspension into the central part of folded leaves (fig. 5). The resulting infected areas were clearly visible allowing accurate comparisons and quick ratings for resistance (fig. 6). Special types of resistance can be recognized (fig. 7).

Figure 4.--High-pressure hand sprayers for field inoculation.

It may sometimes be necessary to grow the greenhouse plants in isolated compartments to prevent infection of rust pustules by Darluca filum (Biv.) Cast., which interferes with ratings.

Individual Races Tested

Testing of individual races of leaf rust in the field is tedious and slow. Greenhouse tests are considerably simpler and faster. All accessions that showed resistance to the mixture of rust races in the field were retested in the greenhouse with individual races. Isolated compartments are necessary to prevent contamination. At Ames, local races R_1 through R_6 were used by the method of Hooker and Russell (1962).

Leaf Blight

Inoculum of leaf blight was gathered in the late fall from a heavily infected cornfield. Leaves with blight lesions were bagged, air-dried, and placed in an unheated room overwinter. Dry leaves were ground in a mill and kept in paper bags until inoculation.

Inoculation of leaf blight was initiated at the tasseling stage or a little earlier at the five- to seven-leaf stage. Since heavy blight development tends to interfere with rust evaluation, blight inoculations should begin when rust reaches its maximum development. The method of Elliott and Jenkins (1946) was followed, but it was modified because hand application was better than spraying. The plants were dusted with inoculum on the check half of the row during previous leaf rust inoculations (see fig. 2). The inoculation was repeated at weekly intervals, usually four or six times, until the corn leaves of susceptible accessions became covered with blight lesions.

Evaluations were made several times after infection by comparing the inoculated part of each row with the uninoculated part. The latter showed the natural infection of blight in the field, if any. Since degree of resistance to leaf blight can be adequately determined by field tests alone, the greenhouse tests are unnecessary. A rating scale of 0-5 was used, as described by Ullstrup (1952) and Ullstrup and Miles (1957).

Diplodia Stalk Rot

Diplodia stalk rot occurs late in the season. Inoculum of this disease is therefore one of the last to be applied. The disease becomes evident soon after pollination and increases in severity as the plants mature (fig. 8). If made at the right time, one inoculation at the third internode is

Figure 5.--Medical syringe used to inject corn plants with leaf rust spores in greenhouse.

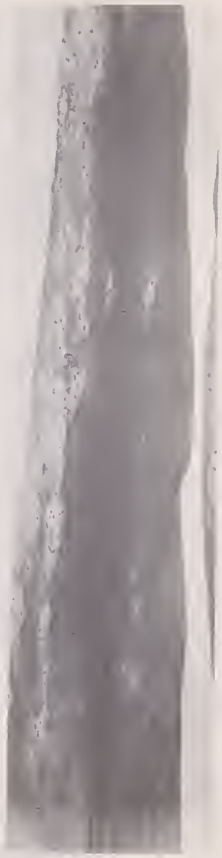

Figure 6.--Restricted area of rust pustules on corn leaves around injection holes (see white spots).

satisfactory. However, in all tests at Ames, 10 plants of each accession were inoculated twice. Early maturing corn was inoculated early in the season and the late maturing accessions later.

Inoculum was prepared by growing the fungus on sterilized oats in Erlenmeyer flasks, washing off the spores with distilled water, and filtering through cheesecloth. The concentrated spore mass was diluted with distilled water and mixed in an electric blender to a light grayish suspension. Inoculations were made by using specially constructed injectors (fig. 9).

Ratings based on the extent of discoloration of the stalk interior were made as late in the season as possible. Stalks were scored from 1 to 6, according to Foley's (1960) scale. In resistant plants only small areas around the injection holes were dark; in susceptible plants the entire internode and in very susceptible plants the injected and neighboring internodes were brownish black and rotten (fig. 10).

Fusarium Stalk Rot

The Fusarium stage of Gibberella spp. is the cause of the dominant type of stalk rot in Iowa. It may cause stalk breaking of corn plants in the fields (fig. 11) alone or more frequently in association with Diplodia stalk rot. Because of its wide distribution in fields, artificial inoculation is hardly necessary. There was little difference in ratings between inoculated and uninoculated plants.

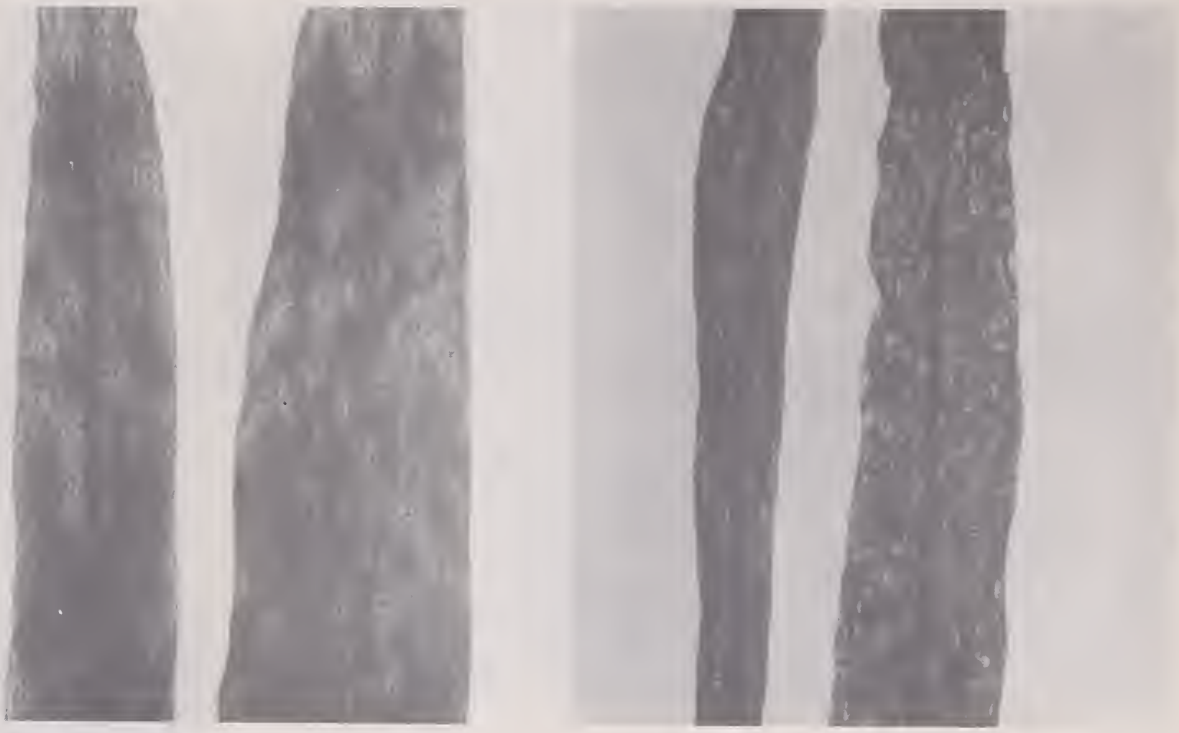

Figure 7.--Heavily infected leaves of susceptible corn accession showing numerous rust pustules (left) and special type of resistance with small lesions around pustules (right).

Ikenberry (1961) made an extensive study of corn kernels and found all samples infected with Fusarium spp. The damage this pathogen actually causes is determined partly by natural resistance in the plants and partly by soil and weather conditions.

Inoculations were made as for Diplodia stalk rot tests. The left half of the row was inoculated and the right half was used as a check.

Ratings of Fusarium stalk rot were made together with those of Diplodia and in a similar manner. Damaged parts were distinguished from one another by the color of rotted tissue and other symptoms (fig. 12).

European Corn Borer

Since this pest was introduced into America from Europe about 1915, American corn varieties have been exposed a relatively short time to its selective activity. They have not yet exhibited genuine resistance. However, various corn accessions react differently to this pest, and breeding work for resistance shows promise.

The first three plants of every row were inoculated once with the borer in early midsummer. Soon borers had reproduced in sufficient number to permit adequate assessment of resistance. Inoculations and evaluations were made in cooperation with the European Corn Borer Research Laboratory, Agricultural Research Service, in Ankeny, Iowa.

Figure 8.--Corn accession heavily infected by Diplodia stalk rot (right), Ames, Iowa, September 1963.

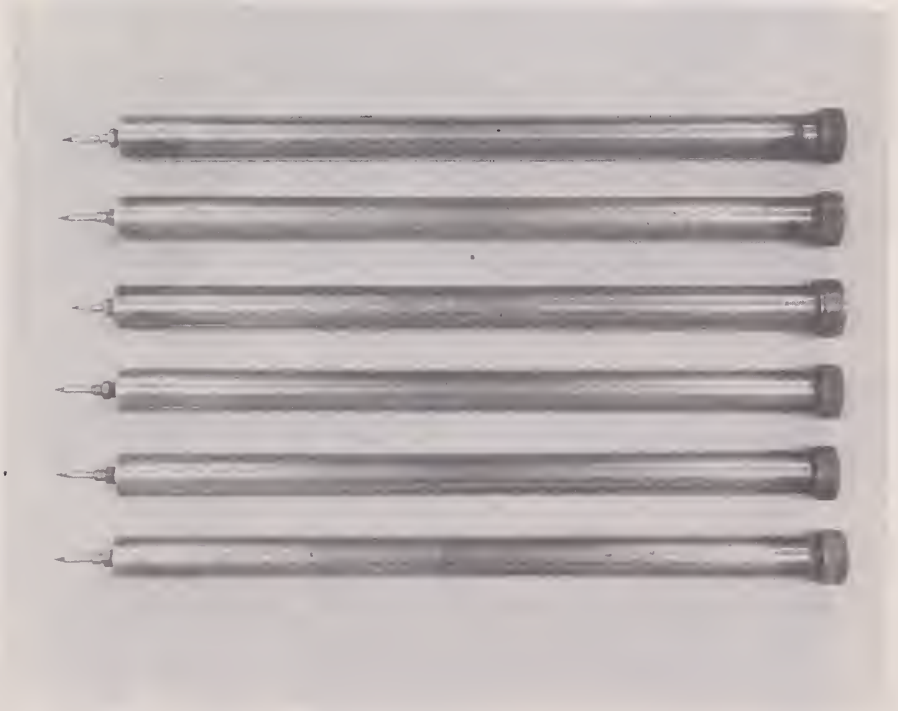

Figure 9.--Injectors for inoculating corn with stalk rot spores.

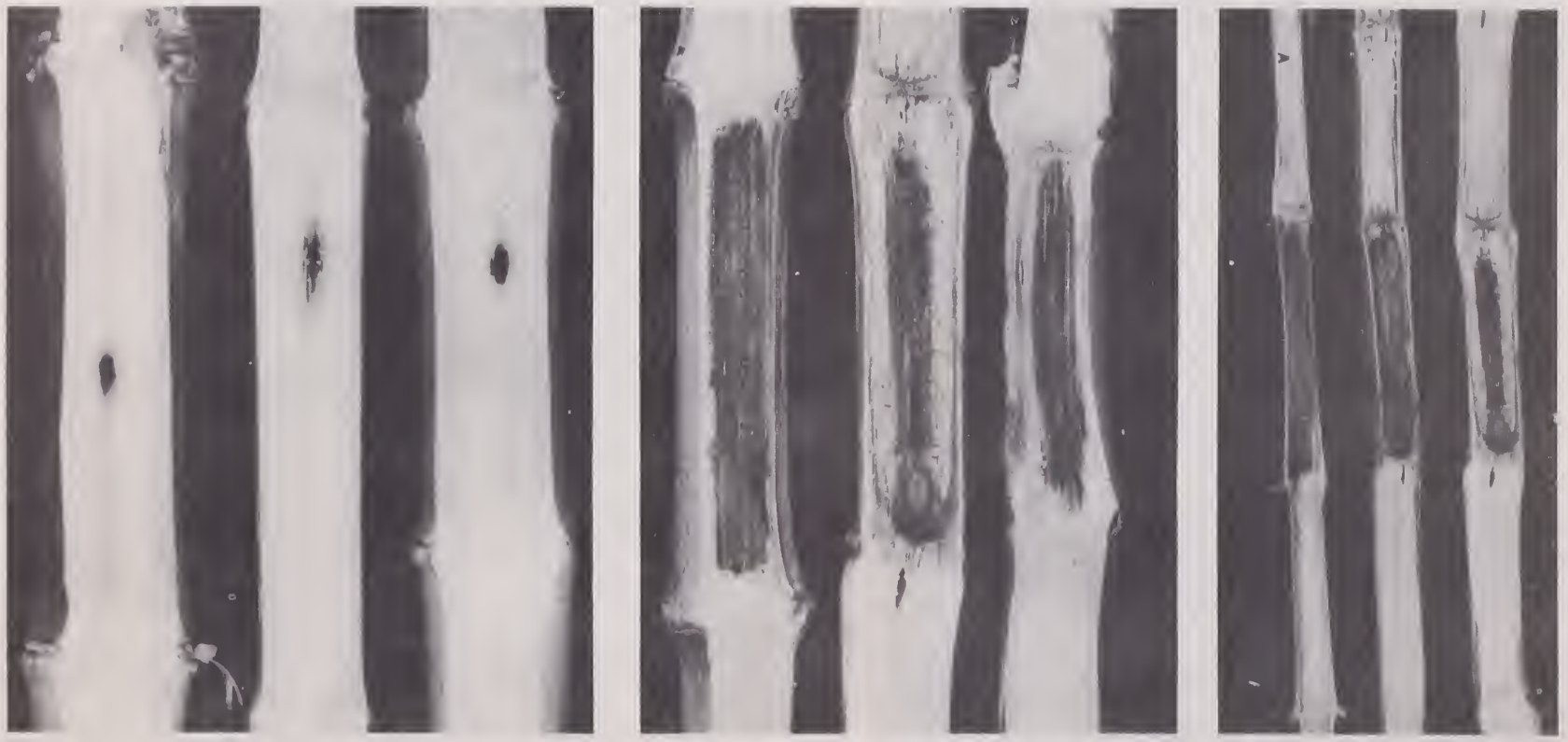

Figure 10.--Diplodia stalk rot-infected accessions: Left, resistant, with small black areas around injection holes; center, susceptible, with large brownish-black areas around injection holes; right, very susceptible, with fungus penetrating into neighboring internodes.

Figure 11.--Corn accession heavily infected by Fusarium stalk rot (left), Ames, Iowa, September 1963.

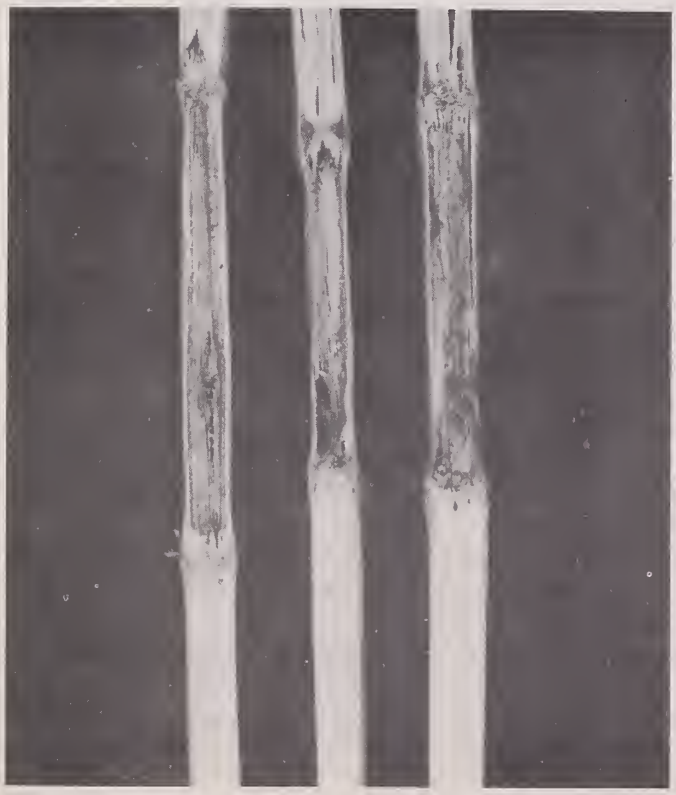

Figure 12.--Very susceptible Fusarium stalk rot-infected accession with rotten internodes, which are lighter color than Diplodia rot-infected accessions.

EQUIPMENT

For the previously described screening, a permanent set of special equipment is preferable. Some instruments needed for a team of five or six workers are as follows:

(1) Vacuum pump for collecting rust spores from greenhouse infected corn plants.

(2) Electric blender for preparing spore suspension for field inoculations.

(3) High-pressure hand sprayers for field inoculations with rust spores (fig. 4). Filling cans is time consuming. Therefore, at least 12 or more are needed for a team of 5 or 6 workers. A supply of compressed air should be conveniently available for refills.

(4) Medical syringes for inoculating greenhouse plants (fig. 5). The 5-cc syringes, with automatic suction devices for filling from bottles, are preferred.

(5) Corn injectors made from 1- by 24-foot brass tubes provided with a sharp steel needle for inoculations with stalk rot spores (fig. 9). These instruments must be shop-built to order. Needles must have a hole about 1 mm. in diameter, through which a droplet of spore suspension can be pushed into the cornstalk. For a team of five or six workers, a dozen such injectors with a reserve of needles should be available. They can be filled in the field.

(6) Corn knives, machetes, or similar large knives used for splitting stalks for stalk rot evaluations.

DISCUSSION

Similar comparative tests with several diseases were reported by DeVay et al. (1957) in Minnesota, but by different methods. These workers evaluated the resistance of inbred corn lines and various experimental crosses to preemergence seedling rot, stalk rot, ear rot, northern leaf blight, and smut. At Ames the soil inoculation method of Schroeder et al. (1953) and the toothpick method of Young (1943) were modified for the large-scale field plantings.

An obvious advantage of the technique used at Ames is the possibility of establishing the combined reaction of plants to several pathogens and insects to which these plants are subjected in the field in a given district. Frequently one pathogen or insect may weaken the resistance of plants to other pathogens or insects. The combined reaction of plants to multiple infection often differs from the damage caused by a single pathogen.

Multiple resistance develops naturally in plants exposed to certain pathogens and insects over a long period. At Ames, therefore, several American corn varieties naturally showed a certain degree of resistance to several American diseases, such as smut, leaf rust, leaf blight, and stalk rot. But there was no parallelism in these varieties in their resistance to foreign diseases and to the European corn borer.

Sometimes symptoms of virus or parasitic diseases are masked by nonpathogenic heritable factors, such as yellow mottle of corn (fig. 1). Small necrotic spots on leaves resemble the young rust pustules or mosaic virus; however, the spots of nonpathogenic yellow mottle are more regularly distributed over the leaf surface than are the pathogenic symptoms.

At Ames about 2,000 corn accessions have been screened by the field method described, and many have been retested in the greenhouse. In addition to known sources of resistance, which have been retested, some new sources were found. Some accessions failed to show resistance previously ascribed to them and were reevaluated.

The corn tested at Ames is but a part of the total U.S. seed collection, much of which remains to be tested systematically. Appearance of new pests, such as the maize dwarf mosaic virus, could make the retesting of all the material necessary.

The incidence and relative importance of pathogens and insects may change in different localities and thus necessitate certain flexibility in this screening method. In other localities pathogens not considered important enough for resistance screening may be deleted and others added.

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